determination enigmatic at this time. Some puzzles remain with respect to comparisons between E16 and E14. We are additionally investigating the peptide-lipid behavior when Glu is introduced in position 12, at the peptide center.

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Influence of pH and Histidine Residues on Membrane-Spanning Helical Peptides

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Synthetic model peptides such as GWALP23 (acetyl-GGALW^{5}LALALALALALW^{19}LAGA-amide)provide a useful host framework for investigations of the influence of polar amino acids, for example histidine residues, within the hydrophobic core of a transmembrane helix. Importantly, membrane-spanning GWALP23 is quite sensitive to single-residue replacements, in part because the transmembrane helix exhibits only limited dynamic averaging of solid-state NMR observables such as the ^{2}H quadrupolar splitting (Biophys. J. 101, 2939). We inserted His residues into position 12 and/or 13 of GWALP23 (replacing either L12 or A13) and incorporated specific ^{2}H-Ala labels within the helical core sequence. Solid-state ^{2}H NMR spectra of GWALP23-H12 reveal a marked difference in peptide behavior between acidic and neutral pH conditions. At neutral pH, GWALP23-H12 exhibits a well-defined tilted transmembrane orientation in both DOPC and DLPC bilayer membranes. To prevent the acid catalyzed degradation of lipids, we employed ether-linked DOPC bilayers to observe the effect of low pH on the L12H mutant. Under acidic conditions GWALP23-H12 is highly dynamic and exhibits multiple states. Indeed, the multi-state behavior of GWALP23-H12, when His is charged between pH 1.5 and pH 3, resembles closely that of GWALP23-R12 at neutral pH (J. Am. Chem. Soc. 132, 5803). The dramatic change in the behavior of GWALP23-H12 indicates a pK_a value less than 3 for His near the center of a lipid bilayer. Investigations are in progress to chemically exchange the C2 imidazole hydrogen of His for deuterium in the peptide, toward a goal of enabling direct observation of the His ring by solid-state ^{2}H NMR over a range of pH and buffer conditions.

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Molecular Insight for the Effect of Lipid Raft on Thrombospondin-1 and Calreticulin Interactions

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Thrombospondin-1 (TSP1) binding to calreticulin (CRT) on the cell surface stimulates association of CRT with LDL receptor-related protein (LRP1) to signal focal adhesion disassembly and engagement of cellular activities (J. Biol. Chem. 275:36358-68, 2000; J. Biol. Chem. 277: 37219-28, 2002). Study demonstrated that lipid rafts are necessary for TSP1-mediated focal adhesion disassembly (J. Biol. Chem. 279, 23510-16, 2004), but the molecular mechanism of the phenomenon is still unknown. In this study, we investigated the interactions of a lipid bilayer and a lipid raft with CRT and TSP1-CRT complex and their effects on the structural changes of CRT and TSP1-CRT complex via atomically detailed molecular dynamics simulations. The lipid bilayer was modeled as a 1-palmitoyl-2-oleoyl-sn-glycero-3phosphocholine (POPC) bilayer with 1152 lipids. The lipid raft was modeled as a bilayer of POPC lipids mixed with cholesterol (CHOL) (40% of CHOL molecules in the lipid raft) or with both CHOL and sphingomyelin (SM) (the ratio of the number of POPC lipid, CHOL and SM is: 3:4:3). Results showed that TSP1 binding to CRT resulted in a more ''open'' conformation for CRT P-domain with respect to the CRT N-domain compared to that of single CRT in a lipid raft environment, but not in a POPC bilayer environment. Sphingomyelin enhanced the ''open'' CRT conformation by TSP1, which could expose the potential binding site(s) in CRT for binding to LRP1 to signal focal adhesion disassembly. Results also showed that micro- and mesoscopic properties of a lipid raft were significantly different from a POPC bilayer, which could also affect cell surface CRT interactions with TSP1. Results from this study provided molecular insight for the effect of lipid raft on TSP-CRT interactions and CRT-mediated focal adhesion disassembly.

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Analysis of the Molecular Organization of Lipoprotein-Associated Apolipoprotein E, an Anti-Atherogenic Protein

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Human apolipoprotein E3 (apoE3) is a 299 residue exchangeable apolipoprotein that has the ability to exist in lipid-free and lipoprotein-bound states. It is composed of an N-terminal domain bearing LDL-receptor binding sites and a C-terminal (CT) domain bearing high-affinity lipid-binding sites; the latter is also responsible for mediating cellular cholesterol efflux. ApoE plays a crucial role as an anti-atherogenic agent in atherosclerosis by virtue of its ability to mediate reverse cholesterol transport by promoting cholesterol efflux from macrophages, a process that leads to the initial formation of nascent discoidal high-density lipoproteins (nHDL). The objective of this study is to understand the molecular organization and conformation of apoE3 (1-299) and isolated CT domain (201-299) on reconstituted HDL (rHDL) and nHDL generated by macrophages. To accomplish this, we over-expressed and purified recombinant apoE3 and the CT domain bearing single Cys at selected sites. rHDL was prepared using unlabeled or pyrene-labeled single Cys variants and phospholipids, while nHDL was generated by exposing cholesterol-loaded J774 macrophages to unlabeled or labeled apoE bearing probes at defined sites. Lipid-associated apoE was obtained by density gradient ultracentrifugation. The relative organization of apoE molecules on rHDL and nHDL was followed by monitoring pyrene excimer fluorescence and by site-specific cross-linking studies, both complementary validators of spatial proximity at ~10 Å distance. The appearance of excimer emission and cross-linked dimers regardless of the location of the single Cys is indicative of pairs of apoE3 molecules oriented parallel to each other on the HDL to form a dimer. Based on our results, we propose that apoE molecules circumscribe a bilayer of lipids adopting a belt-like conformation. Our study offers mechanistic details of apoE interaction with lipids, which aids in understanding its role in cardiovascular disease.

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Dual-Color Fluorescence Cross-Correlation Spectroscopy of Reconstituted Protein-Membrane Systems

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Liposomes are commonly used as model membranes for studying the function of isolated membrane proteins. They are also used for reconstituting large membrane-associated protein complexes, such as the protein coats of intracellular transport vesicles. Here, we show examples of how dual-color fluorescence cross-correlation spectroscopy (or, more generally, fluorescence fluctuation spectroscopy) can serve to optimize in vitro reconstitutions and unravel protein-membrane interactions:

(A) The reconstitution of detergent-solubilized, purified membrane proteins into liposomes is a prerequisite for a myriad of experimental studies, but experimental conditions generally have to be determined on a case-by-case basis. Fluorescence correlation spectroscopy (FCS) distinguishes micelles, liposomes and aggregates in homogeneous and heterogeneous samples based on their different mobilities. Dual-color fluorescence cross-correlation spectroscopy (dcFCCS), an extension of FCS, additionally detects the co-localization of protein and lipid in these diffusing entities, facilitating the optimization process [1]. The principle is not limited to proteoliposome formation, but can be applied more generally to reconstitutions into small diffusing membrane entities, such as liposomes or nanodiscs.

(B) Studies of large, multi-subunit protein complexes, such as the COPII transport vesicle coat, require analysis of protein-lipid as well as protein-protein interactions. By virtue of their low perturbation, fluorescence techniques help optimizing complex reconstitution systems to obtain suitable preparations for cryo electron microscopy analysis of a complex structure [2], and help expanding the complexity of the reconstitution. The interaction of COPII components among each other and with model membranes are investigated.

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Divalent Cation- and Cholesterol-Induced Perturbation in Lipid Lateral Organizations and Polyphosphoinositide-Protein Interactions

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Phosphatidylinositol 4,5-bisphosphate (PIP2) controls many important cellular events. A major challenge in understanding how PIP2 function in vivo is to define its physical state and lateral organization in cell membranes. The hypothesis that PIP2 forms nano-sized clusters in the presence of intracellular divalent cations by electrostatic interactions was examined in model membranes with or without cholesterol-mediated phase segregation. Additional studies show how